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DESIGN OF MIMOTOPES OF A CONSERVED EPITOPE IN DENGUE AND ZIKA VIRUSES FOR THE OBTENTION OF BROADLY NEUTRALIZING ANTIBODIES

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Zika and dengue viruses are members of the Flavivirus genus that share many structural and pathological characteristics. They cause mild fever, rash and general body pain but can cause severe reactions, as hemorrhages (dengue virus), congenital syndrome (Zika virus), or even death. After an infection, virus-specific antibodies are generated by the immune system; however, because of the structural similarity between these viruses, some antibodies can cross-react with different members of the flavivirus family. After a secondary infection, the cross-reactive antibodies can lead to the more severe forms of the disease, through a mechanism named antibody-dependent enhancement of infection (ADE). Recently, some broadly neutralizing antibodies (antibodies that neutralize both, dengue and Zika, viruses), have been isolated and it has been demonstrated that they do not induce ADE. These antibodies are directed to a discontinuous quaternary epitope named the Envelope Dimer Epitope (EDE)[1], located in the envelope (E) protein of both viruses. To obtain EDE, it is necessary to express the complete E protein, which contains other epitopes that induce ADE. This study aims to generate peptides that emulate the EDE epitope structure (mimotopes) without inducing ADE, and study its capacity to elicit broadly neutralizing antibodies against dengue and Zika viruses, to obtain a vaccine candidate for both viruses.

Using the broadly neutralizing antibody EDE1-C8, that does not induce ADE at high titers [2,3], and a phage display library, we identified three peptides that are recognized by EDE1-C8, but share very low or no identity with the E protein sequence from dengue and Zika viruses. Thus, they are probably emulating the structure of the EDE epitope and are EDE mimotopes. The analysis by circular dichroism of the free peptides showed mainly a random coil folding, characterized by a lack of secondary structure. EDE1-C8 did not recognize these mimotopes attached chemically to a carrier protein, and free or phage-displayed peptides did not induce antibodies against native viruses when administered to mice. To improve the stability and folding of the peptides, we designed and produced adeno-associated virus-like particles (VLPs) that display the mimotopes on their surface. VLPs were obtained using the baculovirus-insect cell system, with similar size and structure to the native adeno-associated virus, as determined by cryo-electron microscopy. The modified VLPs were recognized by the EDE1-C8 antibody in a native Dot-Blot, but not under denaturing conditions, demonstrating that the three-dimensional structural conformation of the peptides displayed in the VLPs can mimetize the EDE epitope. The VLPs displaying mimotope 2, in combination with an adjuvant, elicited antibodies against Zika and dengue viruses when applied to mice.

Here, we demonstrate that it is possible to emulate a very conserved epitope in dengue and Zika viruses, with non-related to protein E peptides. This information is valuable for the design of a safer dengue and Zika vaccine without ADE. More research is necessary and undergoing to study the neutralizing and ADE effect of the antibodies produced in response to the immunization with these VLPs-displayed mimotopes.

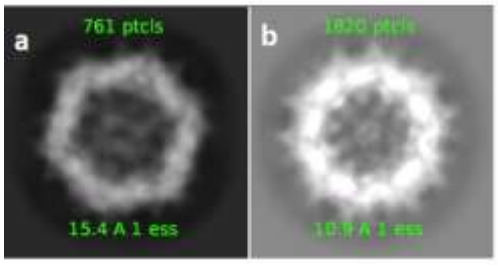

Figure 1- VLPs displaying a control peptide (a) or mimotope 2 (b).

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